Alpine Entomology 5 2021, 101–106 | DOI 10.3897/alpento.5.76930

PENSOFT

Alpine Entomology

Relict populations of *Lyonetia ledi* Wocke, 1859 (Lepidoptera, Lyonetiidae) from the Alps indicate postglacial host-plant shift to the famous Alpenrose (*Rhododendron ferrugineum* L.)

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http://zoobank.org/5AA2883F-4377-4C94-BD91-6D7938872A26

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Academic editor: Thibault Lachat ♦ **Received** 22 October 2021 ♦ **Accepted** 12 November 2021 ♦ **Published** 22 November 2021

Abstract

Lyonetia ledi Wocke, 1859 (Lyonetiidae), was hitherto considered as a boreal species with a circumpolar distribution pattern and relict populations in isolated peat bogs north-east of the Alps (Austria, Czech Republic, Germany). In Europe it is known as a leaf-miner on *Rhododendron tomentosum* Stokes ex Harmaja (Ericaceae) as the primary host-plant and also *Myrica gale* L. (Myricaceae). The first record of *L. ledi* from the Swiss Alps on *Rhododendron ferrugineum* L., the famous Alpenrose, indicates an ancient host-plant switch during postglacial periods when *R. tomentosum* and *R. ferrugineum* shared habitat in the prealps. Conspecificity with northern populations is supported by the adult morphology and supplementing DNA barcodes (mtDNA COI gene). *L. ledi* is the first obligatory leaf-mining species on *R. ferrugineum*. Details of the life-history and habitat are described and figured. The record finally substantiates the probability of an autochthonous population in Carinthia (Austria), from where the species was recently published as new to the Alps.

Key Words

Lyonetia ledi, new host-plant, new record, faunistics, Switzerland, boreo-montane, circumpolar, DNA barcoding

Introduction

Although new national records are of considerable faunistic interest in Europe *per se*, they are often disregarded of only limited scientific value. Here we report an exceptional observation of a species of microlepidoptera which is not only of faunistic but also of considerable ecological interest as the first specialized leaf-miner on *Rhododendron ferrugineum*. The species discussed, *Lyonetia ledi*, was previously known in the Alps only from a single specimen from Carinthia, whose origin seemed questionable, especially because the known host-plants *Rhododendron tomentosum* (Ericaceae) and *Myrica gale* (Myricaceae) are absent from that area. On a visit to the Lower Engadine, Switzerland, on the 29th of July 2021 as part of the surveys for Biodiversity Monitoring Switzerland (Koordinationsstelle BDM 2014), occupied mines of an unidentified species were discovered on *Rhododendron ferrugineum* by PH and subsequently confirmed by JS. The find was immediately surprising, as no specialized leaf-miners were previously known from this plant (Hering 1957) and therefore usually this plant remains disregarded by lepidopterists. The suspicion that it was a member of the family Nepticulidae was rejected from larval habits. The final instar larva made, after leaving the leaf-mine, a spinning typical of the genus *Lyonetia* with the pupa suspended by silken threads. The subsequently emerged moths were identified as *Lyonetia ledi* both according to external morphological features and by subsequent DNA barcoding.

Material and methods

A total of 19 male and 6 female specimens of *Lyonetia ledi* from Switzerland have been examined. Material is preserved in the research collection of Tiroler

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Landesmuseum Ferdinandeum (Hall, Austria) and of Jürg Schmid (Ilanz, Switzerland). Material is either pinned or alternatively set traditionally.

Species identification was based firstly on phenotypic characteristics of adults (wing markings, colour, size) using comparative collection specimens of *L. ledi* from the Tyrolean State Museums as well as on available online illustrations (Rennwald and Rodeland 2021). Despite considerable individual variation, sometimes with complete reduction of dark markings in the basal two-thirds of the forewing, *L. ledi* cannot be confused with any European congeners. Specific characteristics of the male and female genitalia are described and illustrated by Bengtsson and Johansson (2011), for example, but were not examined in the context of the present study. Finally, three samples were used for molecular analysis.

Tissue samples (a single hind leg) from 3 specimens of the suspected *Lyonetia ledi* were prepared according to prescribed standards to obtain DNA barcode sequences of a 658 base-pair long segment of the mitochondrial COI gene (cytochrome c oxidase subunit 1) and successfully processed at the Canadian Centre for DNA Barcoding (CCDB, Biodiversity Institute of Ontario, University of Guelph) using the standard high-throughput protocol described in deWaard et al. (2008). We furthermore analysed 49 public supplementary barcode sequences > 500 bp from BOLD, covering all four of the other European species of the genus: *L. pulverulentella* Zeller, 1839, *L. prunifoliella* (Hübner, 1796), *L. padifoliella* (Hübner, 1813) and *L. clerkella* (Linnaeus, 1758).

All sequences were submitted to GenBank, and further details including complete voucher data and images can be accessed in the public dataset "Lepidoptera of the Alps - *Lyonetia ledi* [DS-LYONLEDI]" https://doi.org/10.5883/DS-LYONLEDI in the Barcode of Life Data Systems BOLD (Ratnasingham and Hebert 2007). Degrees of intra- and interspecific variation of DNA barcode fragments were calculated using the Kimura two-parameter model on the platform of BOLD systems v. 4.0. (http://www.boldsystems.org). A COI Neighbor-Joining tree was constructed using under the Kimura two-parameter model in MEGA 6 (Tamura et al. 2013). A three-letter code (ISO 3166-1 alpha-3, https://en.wikipedia.org/wiki/ISO_3166-1_alpha-3) was used to abbreviate country names.

Results

Molecular analysis

The length of obtained DNA barcodes in three Swiss specimens of *L. ledi* was 586, 632 and 658 bp respectively. A considerable intraspecific divergence was detected in DNA barcodes of *L. ledi* (Fig. 1). European populations and four Canadian *L. ledi* specimens formed one cluster and assigned to BIN BOLD:AAE8230 (n=14), with a mean intraspecific distance of 0.39% and a maximum divergence of 1.22% but 2.24% to the nearest neighbour. However, except for a single weakly deviating sequence from Lower Austria, all specimens from Europe including Swiss samples share the same haplotype, whereas Canadian samples belong to a distinct haplotype. Two additional BINs which include the nearest neighbour of European *L. ledi* from Canada and a unique sequence from South Korea may suggest potential cryptic diversity. Similarly, the considerable intraspecific variability (6.65%) detected in *L. clerkella* in Europe (table 1) must also be tested for potential cryptic diversity.

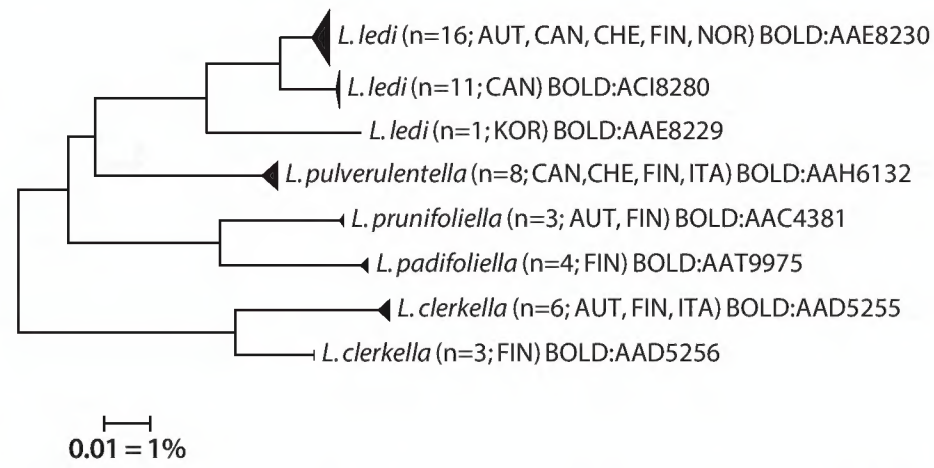
L. ledi (n=16; AUT, CAN, CHE, FIN, NOR) BOLD:AAE8230
L. ledi (n=11; CAN) BOLD:ACI8280
L. ledi (n=1; KOR) BOLD:AAE8229
L. pulverulentella (n=8; CAN,CHE, FIN, ITA) BOLD:AAH6132
L. prunifoliella (n=3; AUT, FIN) BOLD:AAC4381
L. padifoliella (n=4; FIN) BOLD:AAT9975
L. clerkella (n=6; AUT, FIN, ITA) BOLD:AAD5255
L. clerkella (n=3; FIN) BOLD:AAD5256
0.01 = 1%

Figure 1. COI Neighbor-Joining tree of species in the studied *Lyonetia*. Note: the scale bar only applies to internal branches between species. Width of triangles represent sample size, depth the genetic variation within the cluster.

However, apart from exceptions, DNA barcode distances to nearest neighbours are much higher than intraspecific divergence in European *Lyonetia* ranging from c. 6% to 12% (Table 1).

Table 1. Intra- and interspecific divergence in the studied *Lyonetia* species (%).

Species	Mean Intra-Sp	Max Intra-Sp	Nearest Species	Distance to NN
Lyonetia clerkella	2.69	5.49	*Lyonetia ledi*	12.13
Lyonetia ledi	1.68	6.65	*Lyonetia pulverulentella*	8.76
Lyonetia padifoliella	0.43	0.79	*Lyonetia prunifoliella*	5.75
Lyonetia prunifoliella	0.16	0.17	*Lyonetia padifoliella*	5.75
Lyonetia pulverulentella	0.54	1.2	*Lyonetia ledi*	8.76

Alpine population of *Lyonetia ledi*

Material examined. 1♂: Switzerland, Graubünden, Ardez, SE Sur En, 1760 m, 46°45'38"N, 10°11'11.7"E, 6.8.2021 ex larva (*Rhododendron ferrugineum*), leg. Huemer; 3♂: same data, but DNA Barcode TLMF 30911, DNA Barcode TLMF 30912, DNA Barcode TLMF 30913; 4♂: same data, but 21.8.2021 ex pupae, all leg Huemer coll. TLMF. 11♂, 6♀ same locality, 8.2021 e.l., e.p. leg. et coll. JS.

Adult (Figs 2, 3). Head, tuft, and eye-cap as well as thorax glossy white; labial palpus white with some fuscous on outer surface; antenna about length of forewing, pale grey. Wingspan c. 7–9 mm; forewings glossy white; short oblique dark brown streak from tornus at half-length of wing, longitudinal patch at about 2/3, two dorsal streaks from tornus converging in disc at 3/4;

Figures 2, 3. *Lyonetia ledi* adults resting on leaves of *Rhododendron ferrugineum* (Switzerland, Graubünden, Ardez).

Figures 4, 5. Leaf-mines of *Lyonetia ledi* on *Rhododendron ferrugineum* (Switzerland, Graubünden, Ardez).

large ochre-brown patch in distal fifth; costal ciliae with four blackish strigulae, large black apical dot followed by black line and apical scale-pencil. Hindwings grey. The variation of forewing markings is considerable, i.e. a short sub-basal streak below fold at about 1/5 or a complete medial streak as figured by Bengtsson and Johansson (2011) could not be observed, whereas the medial and postmedial markings are completely reduced in one specimen.

For exhaustive description of the adults including genitalia of both sexes see Bengtsson and Johansson (2011).

Biology. In Europe, *Lyonetia ledi* is a widespread leaf-miner of *Rhododendron tomentosum* (Ericaceae), but in the northern part of the continent it is also regularly recorded from the unrelated *Myrica gale* (Myricaceae), a species absent from large parts of Central Europe. Larvae of the newly discovered population from the Engadine mine the leaves of *R. ferrugineum*. The egg is laid on the upper side of a leaf. The tunnel-mine initially extends towards the base of the leaf, then turns and continues alongside the leaf rim towards the leaf tip, where a spacious blotch mine is formed. Only current year leaves, recognizable by their green underside are infested, while older leaves with the plant's name-giving rusty underside may contain mines from previous years only (Figs 4, 5). The mine is hardly visible on the underside. Frass is firstly deposited in

Figure 6. Final instar larva of *Lyonetia ledi* on *Rhododendron ferrugineum* (Switzerland, Graubünden, Ardez).

continuous line which later fills the complete tunnel-mine, whereas it is deposited in irregular flawy patches in the botch-mine. According to Kuroko (1964) frass may also be ejected through semicircular lateral slits along the border of the blotch mine on the lower side. The same author also reports the larva moving to a new leaf and starting to produce a new mine, an observation unconfirmed in alpine habitats. The final instar larva is light yellow with a light brown head and a brownish mottled thoracic shield and ca. 4.8 mm long (Fig. 6). On the underside of a nearby

Figures 7, 8. Characteristic cocoon with final instar larva and pupa of *Lyonetia ledi* on *Rhododendron ferrugineum* (Switzerland, Graubünden, Ardez).

Figure 9. Habitat of *Lyonetia ledi* in Engadine/Switzerland with *Rhododendron ferrugineum*.

leaf, it constructs a X-shaped silken scaffold in the center of which it then pupates like in a hammock (Figs 7, 8). The ca. 4.4 mm long pupa can be easily found when examining the underside of mined or adjacent leaves. First larvae were detected on the 29th of July 2021, two weeks later on the 12th of August 2021 only pupae were found. In captivity the adults emerged after about a week to 10 days between 6th and 21st of August. These data suggest hibernation of the adults as it is also reported from Sweden, with a flight period lasting from mid-August to October and again from April to May (Bengtsson and Johansson 2011).

The larvae seem to be regularly infested by parasitic wasps of *Diadegma cf. semiclausum*, (Ichmeumonidae) (barcoded) and an unidentified species of Ichneumonidae as we found a number of their cocoons that were already empty.

Habitat (Fig. 9). *Lyonetia ledi* is considered as a tyrphobiontic species (Spitzer et al. 1996) because it is restricted to peat bogs. The Swiss habitat is completely different and can be characterized as a northern exposed subalpine Larici-Piceetum plant-association dominated by *Larix decidua* Mill. and *Picea abies* (L.) H. Karst and interspersed *Pinus cembra* L., *Betula pendula* Roth and *Alnus alnobetula* (Ehrh.) K. Koch are also present in the adjacent area. This biotope is located in a north-facing, steep avalanche gully at the bottom of which remaining snow masses may persist into early summer and provide unique microclimatic conditions. Furthermore, the most

infested *Rhododendron* shrubs are often rather puny specimens, growing in very shady places and thus unable to produce flowers.

Discussion

In Europe, *Lyonetia ledi* is a leaf-miner mainly of *Rhododendron tomentosum* (formerly *Ledum palustre*) (Ericaceae), whereas in Asia it has also been recorded from *R. dauricum* L., *R. indicum* (L.) Sweet, *R. kaempferi* Planch., *R. mucronulatum* Turcz., *R. occidentale* Torr. & A. Gray, *R. japonicum* (A. Gray) Suringar and *R. pentandrum* (Maxim.) Craven and in North America from *R. albiflorum* Hook. (Kuroko 1964; Robinson et al. 2010), giving a potentially much broader host-plant spectrum. This is also indicated by regular records from the unrelated *Myrica gale* (Myricaceae) in Northern Europe and the now detected records on *R. ferrugineum* in the Alps. In particular *Myrica* includes a weird selection of species feeding otherwise on other plants, e.g. *Coleophora cornutella* Herrich-Schäffer, 1861 (in Finland only on *Myrica*), *C. uliginosella* Glitz, 1872, *C. vacciniella* Herrich-Schäffer, 1861 (Mutanen in litt.), and *Pseudotelphusa paripunctella* (Thunberg, 1794). Interestingly, however, the congeneric species *L. myricella* Kuroko, 1964 from Japan also feeds on *Myrica* and the genus may contain common metabolite plant additives with *R. tomentosum*. In contrast, *R. tomentosum* has a specialized fauna of Lepidoptera, e.g. *Stigmella lediella* (Schleich, 1867), *Coleophora ledi* Stainton, 1860 (also observed on R. canadense (L.) Torr. at the botanical garden in Oulu, Finland, Mutanen in litt.), *Coleophora obscuripalpella* Kanerva, 1941, *Carpatolechia epomidella* (Tengström, 1869) and *Argyroploce lediana* (Linnaeus, 1758). Both hitherto known European host-plants of *L. ledi* are characteristic of peat bogs and show a circumpolar distribution pattern. Whereas *R. tomentosum* is becoming increasingly rare towards the South, with only a few relict populations in peat bogs of Central Europe north of the Alps, *Myrica gale* is completely absent from the southern part of Central Europe. Following its host-plants *L. ledi* is widely distributed from northern Europe to the Far East (Aarvik et al. 2017; Baryshnikova 2019), with an increasingly patchy distribution pattern in northern Central Europe (Spitzer et al. 1996), and isolated populations in southern Czech Republic and northernmost Austria (Lower Austria) (Laštůvka and Liška 2011; Huemer 2013). The newly detected mountainous population of *L. ledi* from Switzerland is highly isolated at a distance of about 440 km from the closest confirmed records in the northern part of Lower Austria (Huemer 2013). However, records of the host-plant *R. tomentosum* have been published from southern Germany (Allgäu) at a distance of only about 100 km from the Swiss population (Dörr 2007). On the other hand, *R. ferrugineum* is known from sub-recent observations on the border of peat bogs of the Allgäu district (Dörr and Lippert 2004; Bundesamt für Naturschutz 2021). At the maximum stage of the last ice-age some 22'000 years BP, the northern rim of the alpine glaciers extended over today's lake Constance and even approached what is now the city of Munich (Seguinot et al. 2018). It may be hypothesized that *Rhododendron ferrugineum* and *R. tomentosum* both coexisted in the glacier's foreland of this region. Once *Lyonetia ledi* had switched to *R. ferrugineum*, it followed its new hostplant with the receding glaciers in the post glacial period into the alpine valleys where it survives to the present day at very specific sites. As the Lower Engadine became ice-free only at around 14'000 years BP, *L. ledi* was not isolated long enough to develop marked genetic differences to other European colonies. If this hypothesis holds true, *L. ledi* has a part in a group of species like *Exaeretia allisella* Stainton, 1849, *Depressaria leucocephala* Snellen, 1884, and possibly *Agonopterix subtakamukui* Lvovsky, 1998 (all Elachistidae, Depressariinae) which postglacially colonized the northeastern part of the Alps from northeastern glacial refugia and are found there in sparse, isolated colonies. In comparison, several mutations in COI gene are already occurring in Canada's populations with 4 diagnostic and 33 partial characters, which must have been separated for much longer, and the divergence compared with European *L. ledi* is already more than 1%. The observed geographical variation of host-plants seems to be a more widespread phenomenon in tyrphobiontic species and has also been recorded e.g. for *Eupithecia gelidata* Möschler, 1860 (Spitzer et al. 1991).

A unique record of *L. ledi* from the southern Alps in the absence of one of the known host-plants remained unexplained (Wieser 2018). The single adult specimen was collected at light nearly 300 km from the next known population of *Lyonetia ledi* in Lower Austria, whereas the nearest relict population of the host-plant from Allgäu is about 250 km way. Thus, if not accidentally introduced, this specimen may indicate another population from an alpine *Rhododendron*. The habitat is a stream bed at about 650 m above sea level with typical alpine alluvial plants on limestone such as *Dryas octopetala* L. and probably also (unchecked) *R. hirsutum* L. as a potential host-plant (Wieser in litt.).

Acknowledgements

The authors would like to thank Marko Mutanen, University of Oulu, Peter Schönswetter, University of Innsbruck, Michael Thalinger, Tiroler Landesmuseen, Innsbruck, and Christian Wieser, Landesmuseum Kärnten, Klagenfurt for valuable information. We furthermore acknowledge the valuable input of the reviewers Natalia Kirichenko (Sukachev Institute of Forest SB RAS, Krasnoyarsk, Russia) and Marko Mutanen (University of Oulu, Finland). Paul D.N. Hebert and the entire team at the Canadian Centre for DNA Barcoding (Guelph, Canada) are acknowledged for continuous support with sequencing work. The study was

furthermore supported by the Promotion of Educational Policies, University and Research Department of the Autonomous Province of Bolzano – South Tyrol with funds for the projects "Genetische Artabgrenzung ausgewählter arktoalpiner und boreomontaner Tiere Südtirols". Last but not least Robert J. Heckford (Plympton, U.K.) is thanked for his careful language proofreading with valuable comments.

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